Rooting of Trifoliate Bean Leaves and Some Possible Uses in Genetics and Breeding of Beans (Phaseolus vulgaris L.)

Mohamed F. Mohamed[1], E. Arnaud-Santana[2], and Dermot P. Coyne[3]. Department of Horticulture, University of Nebraska, Lincoln, Nebraska, USA 68583-0724.

Rooted leaves from Phaseolus have provided physiologists with a useful system to study certain aspects of photosynthesis (3) and root-nodule formation (4). Although Indole acetic acid (IAA) supplements were used to induce rooting, exogenous auxins are unnecessary when culture solution and not water is used (2). In this report we summarize our observations concerning adventitious root induction on the petioles of trifoliate leaves from six common bean cultivars/lines using tap water and without auxin treatments. Some of possible uses of this simple system in genetic studies and breeding programs of beans are discussed.

Experiments and Results: Six common bean cultivars/lines, Bac-6, 'Charlevoix', Montcalm, 'PC-50', 'Venezuela 44', and Xan-159, were used in all experiments. Seeds were planted in clay pots (two seeds/pot) containing mixture of soil, sand, sphagnum moss peat, and vermiculite (2:2:2:1) at three different planting dates, March, May, and September, 1991, in the greenhouse. Seedlings ten days after planting were fertilized every three days with 200 ppm N from a 9:3.5:16.5 (N:P:K) fertilizer containing trace elements. Plants were grown at 25-28C under normal photoperiods (40°51′ N latitude, lincoln, Neb.). Trifoliate leaves were excised from 20-day old plants. A preliminary experiment with trifoliate leaves of different sizes (one-third, two-thirds, and fully expanded) was conducted from March planting date. Some trifoliate leaves for these experiments were excised with the pulvinus. The petioles of trifoliate leaves were immediately inserted in small plastic pots (0.4 liter) containing water-saturated sphagnum moss peat or vermiculite or perlite or mixture of them (1:1:1 by vol.). The pots were kept in plastic trays containing water to height of 3-4 cm. Petioles of trifoliate leaves were also placed in contact with moistened crumpled facial tissues (Kimberly-Clark) in a 2.5 x 9.0 cm tubes. All explant cultures were then incubated under transparent polyethylene plastic cover (0.5 mm) for 5-10 days to maintain high humidity. The cultures were kept at 25-28C under natural light in greenhouse.

All petioles from the two-thirds and fully expanded trifoliate leaves were observed to develop roots 5-7 days after culture on the potting media or in the culture tubes. Root formation on petioles of the younger trifoliate leaves were induced in 80-90% of the explants and was observed 8-10 days after culture. The existence of pulvinus or a portion of node with the leaf-petiole cutting had no inhibitory effects on root initiation. Roots formed on all areas along the petiole of a detached bean trifoliate leaf. The culture medium had no detrimental effects, since we observed similar root induction on a wide range of potting media or on the moistened tissues in culture tubes.

Experiments for root induction on potting mixture were then repeated on the two different planting dates, May and September. These experiments were conducted with two-thirds to fully expanded trifoliate leaves that contained no pulvinus with their petioles. Trifoliate leaf cuttings were lifted gently out from the culture pots to record the number of days to root initiation. Fourteen days after the culture, the root length were measured. Then the roots were separated, dried (60C for 48h) and the root dry weight was recorded. Data were also recorded for the maintenance time of green and viable leaf lamina for the rooted detached trifoliate leaves and for intact trifoliate leaves on growing plants under the same conditions in each planting date.

Table 1. Mean root length and root dry weight fourteen days after culture of detached trifoliate leaves from six bean cultivars/lines on potting medium in greenhouse.

Cultivar/Line	Root length (cm)		Root dry weight (g)/trifoliate leaf	
	May	September	May	September
Bac-6	26.5	25.5	0.28	0.25
'Charlevoix'	25.7	27.1	0.25	0.26
Montcalm	27.4	27.6	0.28	0.31
'PC-50'	25.3	28.6	0.29	0.32
'Venezuela 44'	27.3	26.4	0.29	0.24
Xan-159	20.4	20.6	0.10	0.19
$LSD_{0.05}$	NS	4.6	0.07	0.04

NS Non-significant.

No differences among the six bean cultivars/lines were detected for number of days to initiate roots and the percent

[1]Visiting Scientist from Department of Horticulture, University of Assiut, Egypt. [2]Arroyo Loro Experiment Station, San Juan de la Maguana, Dominican Republic. [3]George Holmes professor.

of detached trifoliate leaves forming roots. Adventitious roots formed on all leaf-petiole cuttings from the six cultivars/lines 5-7 days after culture. These roots grew fast and their length reached, on average, 20 to 28 cm seven days later (Table 1). However, root length of Xan-159 fourteen days after culture was clearly shorter than the other five cultivars/lines, but a significant difference was not detected from May planting date due to large variability for the root length in the error term. Root dry weight of Xan-159 fourteen days after culture was lower in both planting dates than the other five cultivar/line. Detached trifoliate leaves were viable and green for up to 2-3 months. In contrast, intact trifoliate leaves turned yellow and died after 2 weeks (Bac-6), or 3 weeks ('Venezuela 44') , or 4 weeks ('Charlevoix', Montcalm, 'PC-50', and Xan-159).

Discussion: The results obtained here from the six different bean cultivars/lines confirmed previous reports (2,3 and 4) that indicated easy induction of adventitious roots on leaf cuttings from Phaseolus. Also, rooted leaves were observed to remain green for long periods. However, leaves turned yellow 5-7 days after culture of bean detached leaves (4) and it was necessary to spray the lamina with urea or to add ammonium nitrate in the nutrient solution, but the fertilization level and schedule for the explant source plants was not shown. Explants should be affected by the nutrient status of the donor plant. Although, the effect of the fertilization was not studied in our experiments, the induction of strong roots using tap water only and the long time maintenance of green leaves may due to the explants having an adequate nutrients from the donor plants. In his study (4) root formation in beans was inhibited when the pulvinus were exist on the petioles. In contrast, leaf-petiole cutting of soybean should include a portion of the node to provide meristematic tissues for root initiation (1). Unlike these reports, we observed root formation on all areas along the petiole of detached bean trifoliate leaf, regardless of the presence of the pulvinus. If explant donor plants are needed for seed increase, it should be preferable to exclude pulvinus to avoid any damage that may occur. The most critical factor we notice for root induction was maintenance of high humidity until root initiation occured. Therefore, we placed the leaf cultured pots in plastic trays containing extra water. Similarly, even slight ventilation in the cultures of trifoliate-cuttings of soybeans (1) resulted in the death of the cuttings before root formation.

We propose that this simple technique has the following possible applications in genetic studies and breeding programs of common bean:

Chromosome observations. High mitotic indices have been observed in soybean (1) from root tips of detached leaves. The petiole tips of soybean were dipped in 0.3% (#30) IAA powder to speed root production. The simple protocol we described for initiation of roots on trifoliate leaves of common bean in culture tubes avoids the use of plant growth regulators that may have an effects on the dividing chromosomes. This detached leaf-rooting technique may be valuable for cytological investigations on unique individual plants such as from tissue culture. The technique may also be employed when some special pretreatments (to get clear chromosome observations) are used in preparing the growing roots.

Screening for disease resistance. The technique for detached leaf-rooting on potting medium may be used to reduce costs for screening large populations or large number of germplasm especially for reactions to different pathogen strains and under different environmental conditions. It permits testing of the same host-genotype for multiple-disease resistance and avoids any possible physiological interactions that may occur among different pathogens. The maintenance of viable leaves for relatively long time periods offers flexibility of times to run the experiment. The technique also may be beneficial when only limited seeds of new germplasm is available so that it can be increased and simultaneously studied for the reaction of a pathogen. It may also be used in root diseases investigations. We used this technique (5) to evaluate the reaction of common bacterial blight disease [Xanthomonas campestris pv. phaseoli (Smith) Dye] in the six cultivars/lines and similar results were obtained for the rooted leaf cutting in the greenhouse and with intact trifoliate leaves.

Literature Cited

1. Blomgren, S.M., L.A. Amberger, H.E. Heer, and R.C. Palmer. 1988. A petiole-rooting technique for soybean chromosome observation. Soybean Genetics Newsletter. 15:153-154.
2. Gregory, F.C. and B. Samantarai. 1950. Factors concerned in the rooting responses of isolated leaves. J. Expt. Botany 1:159-193.
3. Humphries, E.C. 1963. Dependence of net assimilation rate on root growth of isolated leaves. Ann. Botany 27:175-183.
4. Lie, T.A. 1971. Nodulation of rooted leaves in leguminous plants. Plant & Soil. 34:663-673
5. Mohamed, M.F., E. Arnaud-Santana, and D.P. Coyne. 1992. Reaction of common bacterial blight disease in intact and detached leaves of beans (Phaseolus vulgaris L.). Ann. Rpt. Bean Improv. Coop. 35:(in press).